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BORDEN'S REVIEW of NUTRITION RESEARCH

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THE INFLUENCE OF DIETARY FACTORS ON THE REGENERATION OF BLOOD PLASMA PROTEINS

With the dramatic strides made in the science of nutrition following the discovery of the vitamins, interest has been focused very largely on these vital factors and on the powerful influence they exert upon life processes. It has taken the present war with its attendant evils of hunger and starvation, however, to impress the realization that all is not vitamins in nutrition. Even in the United States the pinch of food shortage is making itself felt and more serious, perhaps, is the growing awareness of a deficiency in protein foods which looms ahead.

When the food supply was plentiful there was little need for concern about meeting protein requirements, but with valuable protein foods such as meat and cheese already on the ration list, more serious consideration must be given not only to the problem of adequate protein supply but to the proper evaluation of the nutritive character of individual protein foods. The curtailment of meat and cheese supplies throws an added burden upon milk, cottage cheese, eggs, fish, peanut and soybean products. Cereals are still plentiful, but the proteins they furnish are not nutritionally complete unless supplemented, for example, with milk proteins.

Proteins and their component amino acids form the very basis of living matter from elemental cell protoplasm to the most intricate and complex tissues and organs. It has been known for some time that certain proteins excel others with respect to their growth-promoting properties and this has served as the basis for their classification. Recent nutrition research, however, has brought to light a vital new function of dietary proteins which has been found to be independent of the old classification of proteins as "biologically complete" or "biologically incomplete." Scientists have found that certain proteins in the diet, irrespective of completeness or incompleteness, exert a profound influence upon the specialized proteins of the blood plasma, maintaining them at normal levels and aiding in their restoration when depleted through malnutrition, disease or actual loss of blood through hemorrhage.

The properties peculiar to those dietary proteins exhibiting superior ability in the formation of plasma proteins are still a matter of investigation. It is signi-

ficant that perhaps the most potent of these superior dietary proteins, *milk lactalbumin,* ranks also as one of the superior complete proteins according to the older classification. Milk casein, beef serum proteins and egg white proteins, all nutritionally complete, have also been found to be potent factors in the production of plasma proteins. On the other hand, certain cereal grain proteins, inadequate from the point of view of biological completeness, also rank high in ability to stimulate the formation of plasma proteins.

The significance of the proteins of the plasma has only recently been more fully recognized. Evidence has been presented that these blood proteins, in addition to their physico-chemical and immunological activities, are intimately involved in the total protein metabolism and nutrition of the body and that they are important not only to the blood, but to other body tissues which depend upon them as a vital source of essential protein material. For this reason, depletion of plasma proteins caused by protein malnutrition such as may occur during periods of food privation or as a result of diets containing protein foods of inferior nutritional quality, or even in as short a period as one week of improper diet, presents a serious hazard to the health of the blood and other tissues of the body and, indeed, to life itself. In view of this fact, the study of plasma proteins, their maintenance and regeneration by dietary means, has assumed especial significance in present-day research.

The Nature of Plasma and of the Plasma Proteins

Blood plasma is the liquid portion of the blood apart from the various suspended red blood cells, white blood cells and platelets. The plasma itself contains considerable dissolved material, about seven percent of which is protein in nature. Functionally, the plasma transports to the body cells amino acids, glucose, vitamins and other nutrients absorbed from the digestive tract and carries away cell excretion products.

The plasma proteins are among the most important constituents of the blood plasma. They consist chiefly of *serum albumin, serum globulin,* and *fibrinogen.* Besides the fact that fibrinogen is concerned with the mechanism of blood clotting*, the plasma proteins are to a large extent responsible for the specific gravity, viscosity and colloid osmotic pressure of blood. The latter is of extreme importance in the regulation of water exchange between the blood stream and the surrounding tissues. Disturbance in the plasma protein concentration causes a significant alteration in the osmotic pressure of the blood resulting either in dehydration or hydration of body tissues. The condition arising from an excess water content of body tissues due to decreased plasma proteins is known as *dropsy* or *nutritional edema* and is characterized by a softness and swelling of the tissues. According to Weech *et al* (1) plasma protein depletion resulting from

*cf. BORDEN'S REVIEW *of* NUTRITION RESEARCH, "*The Story of Vitamin K,*" April 1943.

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protein malnutrition is primarily at the expense of *serum albumin*. Consequently, in the treatment of nutritional edema it is essential that the serum albumin fraction of the plasma be restored to normal levels.

More recently, experiments on the regeneration of plasma proteins have revealed that these constituents of the blood also have an important bearing on the general problem of protein metabolism and that consequently their functions are broader and more significant than heretofore believed.

The Importance of Plasma Proteins in Nutrition

About sixty years ago, Voit and other well-known physiologists, following up the early theories of Mulden, believed that the proteins of the blood plasma had a great deal to do with the nutrition of tissue cells. Years later, with the discovery that the amino acids were absorbed from the intestines and carried throughout the body, attention was centered entirely on the role of these "building stones" in protein metabolism. The concept that plasma proteins were actively concerned in the process was temporarily discarded in favor of the theory that plasma proteins, once formed from the amino acids, are inert substances having nothing to do with tissue nutrition. Their functions were believed limited to activities of a physico-chemical or immunologic nature; that is, plasma proteins were deemed to be essential only for the maintenance of normal osmotic pressure, viscosity, etc. and for participating in defense mechanisms against infection.

Evidence against the theory that plasma proteins are static except in cases of loss by hemorrhage, proteinura (protein loss from the urine in certain kidney disorders), etc., has recently been presented by Whipple and his associates at the University of Rochester (2). They subjected dogs to bleeding, reinjecting the red blood cell component of the blood, so that actually only the plasma and its proteins were reduced. They found, however, that unless the bleedings were performed daily for a prolonged period of time, the animals were able to produce new plasma proteins even though they were *completely deprived of food by mouth*. This meant that there must have been reserve body stores of protein coming from the muscles and other tissues which were being utilized in the formation of new plasma protein. Of course, after a period of continuous plasma depletion by this method (*plasmapheresis*) the reserve stores were used up and the plasma proteins could no longer be replaced by the fasting animal.

In another series of experiments, Whipple and his collaborators were able to keep dogs alive by injecting large amounts of dog plasma into their veins and feeding nothing but sugar by mouth. To their surprise, the large excesses of injected plasma proteins did not escape in the urine nor did the protein concentration of the plasma increase more than 50% of normal. Large amounts of plas-

ma proteins were going in, yet none was leaving. This was indeed puzzling; what was happening to the extra plasma proteins?

A study of the animals' nitrogen balance soon answered this question. It was found that *despite the fact that the dogs were receiving only sugar by mouth —and no protein food—they were being maintained in perfect nitrogen balance merely by injections of blood plasma.* This showed very clearly that the body's nitrogen and protein needs were actually being supplied by the proteins from the injected plasma.

They later found that plasma protein fed by mouth under identical conditions is also utilized to meet body needs. In fact doctors have recently succeeded in keeping alive babies who are unable to tolerate food by administering plasma proteins by mouth (3).

Many additional facts serve to substantiate the theory that the proteins of the blood plasma can be utilized freely in the body economy. For example it is generally known that resistance to various toxins and poisons is largely dependent upon the dietary intake of protein as well as upon a normal concentration of protein in the blood plasma. This is especially important in operative procedure, malnourished patients usually being more susceptible to the ill effects of anesthetics. In the dog it has been found that acute chloroform poisoning may result in severe injury or even destruction of as much as two-thirds of the liver tissue. Davis and Whipple (4) found, however, that *on an intake of sugar alone,* the animals are able to completely regenerate the liver to normal. Furthermore this repair occurs within a short period of from ten to twelve days and represents a very large production of highly specialized liver cell protein which obviously is derived from other body proteins or from the plasma proteins.

From these experiments it is apparent that injected protein in the form of blood plasma is actually utilized by the experimental dog to meet protein needs to the same extent as are food proteins supplied by mouth. If this can happen in an emergency—as represented by protein starvation or total fasting—it is quite likely, according to the investigators, that normally there is also a certain amount of "give and take" between body protein and plasma protein. All of these facts point to a *dynamic equilibrium* between tissue protein and plasma protein depending upon the particular physiological needs of the moment. In the absence of food protein the body can use material coming from one body protein to fabricate badly needed protein material of different character.

The concept of a dynamic equilibrium between body constituents and dietary constituents is a somewhat revolutionary idea and is not restricted to protein metabolism alone, but includes the intricate metabolism of fats, carbohydrates and other nutrients. It was once thought that body tissues, apparently inert stores of depot fat, etc., once formed, remained static and unchanged. It

has since been conclusively demonstrated with the aid of isotopes that all the constituents of the body are in a constant state of flux and that molecules of even the most seemingly inert tissues are continuously being replaced by new molecules from the breakdown of other tissues and from the diet. Schoenheimer, who accomplished a great deal of the pioneer work in the study of intermediate metabolism through the use of isotopes, expresses the principle of dynamic equilibrium in the following excerpt from a highly interesting book on the subject (5):

> "The large and complex molecules and their component units . . . are constantly involved in rapid chemical reactions. Esters, peptides and other linkages open; the fragments thereby liberated merge with those derived from other large molecules, and with those absorbed from the intestinal tract, to form a metabolic pool of components indistinguishable as to origin. . . . Part of the pool of newly formed small molecules constantly reenters vacant places in the large molecules to restore the fats, the proteins, and the nucleoproteins. . . ."

With respect to the principle of dynamic equilibrium as it applies to plasma proteins and their role in total protein metabolism, Madden and Whipple (6) state:

> "The ultimate source of construction materials is food protein furnishing amino acids absorbed from the intestinal tract and synthesized in the liver cells (and elsewhere) into plasma proteins. This same influx of amino acids and/or the plasma proteins, support protein formation throughout the body. A part of the body protein forms a *reserve against adversity* in the sense that it can be circumspectly depleted without apparent injury to the body. The supplies of amino acids coming from outside the body and the demand for protein materials within the body are in a constant state of balance with these protein reserve stores, a *dynamic equilibrium*."

The Influence of Diet on the Regeneration of Plasma Proteins

Studies of nutritional edema as a typical deficiency disease formed the background for present knowledge concerning the significance of diets inadequate in their protein content. Like other deficiency diseases, nutritional edema has appeared in epidemic form at times, associated with conditions of great adversity. Because of this, the disease has come to be known descriptively as war edema, hunger edema, prison edema, camp edema, etc. Shortly after the first World War nutritional edema was widely prevalent throughout Central Europe. Investigation into the nature of the disease invariably disclosed the fact that patients were unusually in a condition of negative nitrogen balance (7). Years later, studies on the disease in the United States led to the same conclusion (8). From this observation, as well as from an analysis of the diets used in regions where the disease existed in epidemic form, it soon became apparent that nutritional edema is the result of diets deficient in protein.

Among the most interesting studies on nutritional edema are those of Weech (9) made during a two-year period in China about fifteen years ago. The large masses of people in North China subsist chiefly upon a diet of cereal grains, millet and corn. Under normal conditions, particularly when the diet is supplemented with native vegetables and the soybean, this diet is just adequate for the maintainence of health. In times of stress, however, and especially when disease is involved, the diet becomes inadequate and protein deficiency soon becomes manifest, as does evidence of other deficiency diseases. Weech found that the most prevalent of such diseases is nutritional edema. An analysis of the blood plasma of twenty-eight patients revealed that there was a marked reduction in the normal level of plasma proteins (from 7% to 3% or less), the most serious disturbance being in the level of *serum albumin*. According to Weech's observations, the serum globulin level was practically unaltered (Fig. 1). High protein diets succeeded in restoring normal levels of serum albumin in the blood plasma with an accompanying disappearance of the edematous condition.

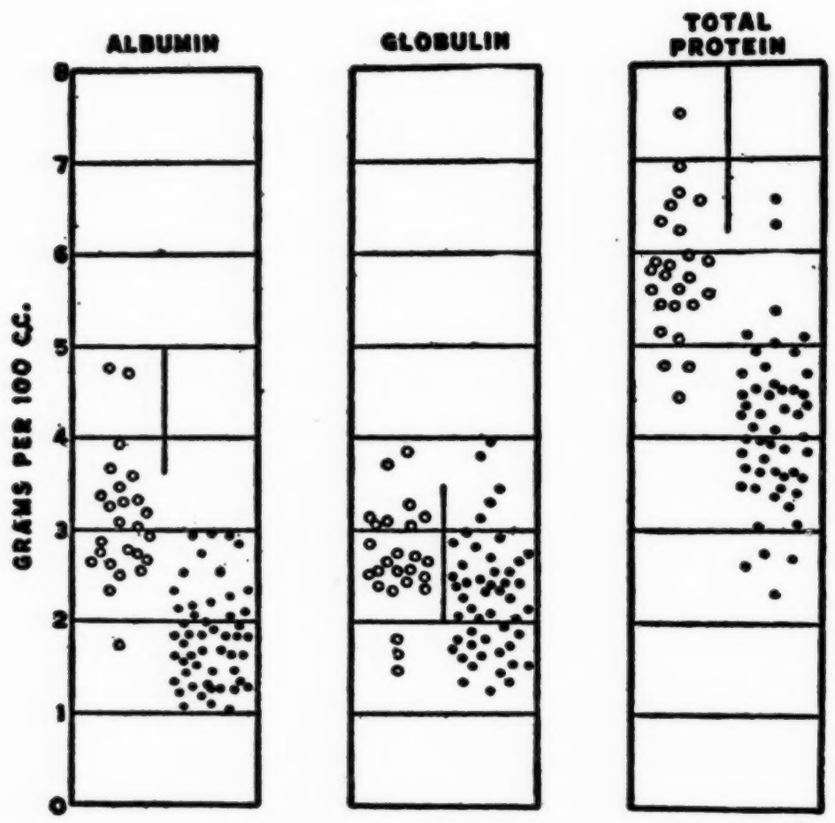

Fig. 1.—Summary of seventy-seven observations of the serum proteins on twenty-eight patients with nutritional edema. The black circles refer to estimations made while edema was still present; the open circles indicate determinations after the disappearance of edema. The vertical lines in the middle of each column show the range of normal variation. (From Weech: Internat. Clin. 2: 223, 1936,)

In 1918, investigators in Whipple's laboratory in Rochester (10) had already found that certain food proteins were distinctly superior to others in their ability to stimulate the formation of new plasma proteins. For many years, these investigators had been studying the problems of anemia and blood hemoglobin regeneration. They had found that liver and kidney, for example, were highly effective for stimulating the formation of new hemoglobin in experimental anemia (11). This led them to believe that liver and kidney would undoubtedly be valuable foods in the regeneration of new plasma proteins as well. To their

surprise, this proved to be untrue. They found, sure enough, that liver was very effective in promoting the formation of new plasma proteins (12), but on the other hand, kidney was of little value in this respect. Likewise they found that milk casein which was not particularly effective in hemoglobin formation, was extremely potent in plasma protein regeneration. In later experiments it was found that certain vegetable or grain proteins, particularly the proteins of the soybean, equalled or excelled various animal proteins in their ability to build new plasma proteins. In fact malnourished Chinese babies who rarely have milk in the diet are kept alive only through the feeding of soybean formulas.

These facts led them to conclude that food proteins cannot be grouped into vegetable, grain, animal, complete or incomplete and their potencies in plasma protein regeneration related to such grouping. They found that it is necessary to test each protein systematically and individually "in the fire of metabolism" in order to ascertain its value in the regeneration of plasma proteins.

Methods for Testing Potency of Dietary Proteins: Whipple and co-workers (13) used the plasmapheresis technique as the basis for determining the value of a food in the regeneration of new plasma proteins. As previously described, they were able with this technique to deplete dogs of plasma proteins through bleeding combined with restoration of the red blood cells to prevent anemia. Following continued daily plasmapheresis, the body stores of reserve protein material are used up, but the normal protein content of the muscles and other tissues is not affected. The plasma protein level is maintained at a concentration just above the edema level by a low protein basal diet. The addition of various protein foods to the basal diet is immediately reflected in an increase in the concentration of blood plasma proteins. This increase varies with different proteins, indicating that some are more potent, gram for gram, than others.

Weech and Goettsch (14) at the College of Physicians and Surgeons, Columbia University, have devised an entirely different procedure for depleting the plasma protein level in experimental dogs. This method was evolved as a result of Weech's early observations in China (9). In his studies there on nutritional edema, it will be remembered, he observed two important facts, namely: (a) a deficit in plasma proteins, particularly *serum albumin,* is the direct cause of edema, and (b) a protein-deficient diet leads to a deficit in plasma proteins. In later studies with Dr. Goettsch at the Babies' Hospital in New York City on malnourished children suffering from nutritional edema, Weech confirmed his observation that protein-poor diets result in a serious depletion of the serum albumin. As a result of these observations, Weech and Goettsch decided to reproduce the disease in experimental dogs by keeping them on a diet *extremely* low in protein. After several attempts, they succeeded in devising a suitable low-protein diet which rendered the animals hypoproteinemic (i.e. deficient in blood proteins) within three weeks. When the animal reaches this stage, the test protein is fed and its effect on the level of serum albumin measured.

A number of such measurements determined for the same food on a number of different dogs yields an *average* value which is termed the "potency value" of the food. The more potent a food, of course, the greater the increase in serum albumin concentration resulting from its ingestion.

In both the plasmapheresis technique of Whipple and the protein starvation technique of Weech, the principle is to render the animal hypoproteinemic and then to measure the effect of the test food on the restoration of either total serum protein or serum albumin*. The two methods differ in that in one case (Whipple *et al*) the plasma proteins are depleted by mechanical bleeding and the muscle proteins remain virtually intact, whereas in the other (Weech *et al*) the plasma proteins—and the muscle reserve proteins as well—are depleted by dietary means closely approximating conditions in human malnutrition or starvation.

Despite the wide variations in the methods employed by these two groups, however, it is significant that they have, for the most part, confirmed each others work with respect to the potencies assigned to various food proteins. Thus, for example, according to both the plasmapheresis technique and the dietary depletion technique, *beef serum, beef muscle* and *milk casein* were all found to be, in the order named, highly potent dietary factors influencing plasma protein regeneration (15). Such close correlation of values derived from widely divergent procedures gives even greater validity to the results obtained.

Beef Serum and Milk Proteins (Lactalbumin and Casein) Among Most Potent Stimulants of Plasma Protein Regeneration

The development of assay techniques for measuring the capacity of various foods to stimulate plasma protein formation led to the discovery that certain proteins are distinctly superior in this respect. At the present stage of investigation, an analysis of values reported by various workers in the field, indicates that perhaps the most potent of all dietary factors for plasma protein formation is the milk protein, lactalbumin. Preliminary clinical tests with lactalbumin have given very favorable results in the treatment of hypoproteinemia associated with edema and also with certain kidney disorders such as nephritis. The recent development of a soluble form of lactalbumin by Supplee of the Borden Biological and Chemical Laboratories has made available a more palatable form of this product which is now being used in a further series of clinical studies. Beef serum proteins have been found to be another highly potent dietary means of stimulating plasma protein formation, but at the present time, this particular product is not widely available. The following table lists results for a few typical proteins tested by the various groups.

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*Since Weech has found that decreased serum albumin is the chief factor in hypoproteinemia, he has justified the assumption that increase in serum albumin levels is virtually equivalent to increase in total plasma protein.

VALUES FOR TYPICAL PROTEINS WITH RESPECT TO PLASMA PROTEIN REGENERATING ABILITY

(Listed in Order of Decreasing Potencies)

ROCHESTER GROUP (12, 13)		COLUMBIA GROUP (15, 16, 17)		YALE GROUP* (18)	
beef serum lactalbumin egg white skeletal muscle smooth muscle	excellent	lactalbumin (soluble) beef serum (dried)	excellent	lactalbumin beef serum protein casein	excellent
casein whole liver liver fractions beef heart	good	egg white cow's colostrum** egg (whole) cow's milk beef chuck beef liver casein	good		
pancreas salmon kidney	poor	wheat gluten gelatin	poor		

*Because of limitations of this review, a detailed description of the method devised at Yale University by Melnick, Cowgill and Burack (19) cannot be given. Briefly, the method is a modified plasmapheresis, combining features of both plasmapheresis and starvation techniques, with certain modifications designed to eliminate supposed inaccuracies in these methods. Here too, it is noteworthy, that despite differences in method, results are again closely correlated with those of other investigators.

**Cow's colostrum is a thick yellow fluid secreted immediately after parturition and precedes the secretion of true milk.

The question naturally arises as to what factors are responsible for the superiority of one protein over another in these experiments. Since in certain instances vegetable proteins may be superior to animal proteins, and incomplete proteins superior to complete ones, it is apparent that the usual classification of proteins is not useful for indicating their effectiveness in plasma protein formation. Madden, Whipple *et al* (20) suggest the possibility that certain amino acids, or combinations thereof, may be the key to the potency of a given protein or combination of proteins. They showed, for example, that feeding gelatin had no effect in restoring lost plasma proteins. However, when the gelatin was supplemented with the amino acid cystine, in combination with either tyrosine or tryptophane, a potency equivalent to that of lactalbumin or beef serum was obtained. When cystine was removed from the combination, the effectiveness of gelatin was lost. (It is interesting to note that, in this combination for example, tyrosine and cystine are two amino acids not essential for growth (21). Nevertheless, *for plasma protein formation,* it is the dispensable amino acids which are necessary, and if they are replaced with their nutritionally essential analogues, phenylalanine and methionine, the plasma protein regenerating ability of the combination is lost.) As a result of additional experiments along these lines, Madden *et al* conclude that *under certain fixed conditions, cystine emerges as a key amino acid in the production of blood plasma proteins.*

It has been suggested (17) that the relatively high cystine content of lactalbumin (22) may, according to the theory of a "key amino acid," very well explain the superiority of lactalbumin as an especially potent protein for stimulating plasma protein regeneration.

Conclusion

The importance of maintaining a normal concentration of protein in the blood plasma has been confirmed by recent experimental investigation indicating that the plasma proteins are intimately involved in total protein metabolism. It has been found that inadequate or poorly balanced diets are frequently deficient in both quantity and quality of protein material and that such deficiency is readily reflected in abnormalities of blood plasma proteins. Not infrequently, restricted diets used in certain disorders such as peptic ulcer, eczema, colitis, etc., may be quite deficient in protein and may result in serious depletion of vital plasma proteins. The possibility of dietary protein inadequacies is further enhanced because of increased food shortages at home and actual food privation abroad.

It is now known that a deficiency in plasma proteins such as may be brought about by any of the circumstances described above, is intimately related to a number of pathological conditions, including edema and nephritis. The recent discovery that specific food proteins (such as the milk protein, lactalbumin, and the proteins of beef serum) are capable of stimulating plasma protein production, presents the possibility of more direct and effective nutritional therapy than has heretofore been possible in the treatment of such disorders.

The vital importance of maintaining plasma proteins at a level of nutritional adequacy imposes, in these times, a new and urgent dietary problem. This problem involves the careful assessment of the nutritive character of protein foods available under present conditions from a dual viewpoint, namely: (1) their biological completeness and (2) their specific ability to influence plasma protein production. From present experimental evidence, as well as from previous nutrition experience, it is apparent that we must rely more than ever upon meat, milk, eggs, and possibly soybean products for those proteins which are vital in the economy of the living organism.

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NEWS DIGEST

ICE CREAM has been found by Navy psychiatrists to be among the important and effective contributory treatments for "combat fatigue", commonly known as shell shock in World War I. The condition is a serious emotional disturbance brought on by tension, repeated air alarms, bombings, lack of sleep, sight of human suffering, etc. It is reported that patients respond favorably to treatment with ice cream and shower baths—two important factors, along with other small comforts, in the effective therapy of combat fatigue. As a result of these observations, ice cream takes a very important place in the diet of our fighting men.

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DEHYDRATED MEAT when cooked, retains much of the B complex vitamin content of the fresh product, according to recent studies. Riboflavin, it was found, is retained completely. Pantothenic acid and niacin are retained to the extent of 70% and 80% respectively. In the case of thiamin, which is less stable to the processing, the retention is approximately 60%. This high retention of the vitamins results in a dry product containing on an equal weight basis about 50% more thiamin than the raw meat and two to three times as much of the other B vitamins, due to the concentration effected by the removal of moisture and excess fat.

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AMERICAN CHEDDAR CHEESE may be dehydrated by a new process which is claimed to be more direct than present commercial drying processes and which, when available for general use, will release essential spray-driers for the dehydration of other types of foods. In the new process, the natural full-fat cheese is grated and then partly dried at room temperature. This causes the fat to be sealed up in the numerous case-hardened particles of cheese curd. The particles are then dried by heated air without loss of the fat. The resulting product is a natural cheese, with full fat content, lacking only the water.

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FLOUR AND FARINA may soon be enriched with vital minerals and vitamins under the Federal Food, Drug and Cosmetic Act. Proposed standards require the addition of thiamin, niacin and riboflavin in all *enriched* flours and farinas. Various types of enriched flour will require the addition of iron; other types will require added calcium. Iron will be required in all enriched farinas; calcium will be an optional ingredient. Minimum and maximum values for vitamins and minerals have been specified for the various products.

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MILK enhanced with naturally inherent vitamins and minerals is a new companion product to vitamin D fortified milk. One quart of the new product contains at least 4,000 units of vitamin A, 400 units of vitamin D, 1 milligram of thiamin, 2 milligrams of riboflavin, and 10 milligrams of niacin. With respect to minerals, there is present in one quart of the fortified milk, 1000 milligrams of calcium, 800 milligrams of phosphorus, 10 milligrams of iron, and 0.1 milligram of iodine. The fortifying materials may be added to ordinary homogenized or non-homogenized milk, before pasteurization. One quart of the new enriched milk supplies the full minimum daily requirements of all of these vitamins and minerals. The enriched milk is already being marketed in several states and it is expected to be made available in other states as the program expands.

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VITAMIN B COMPLEX added to the diets of war workers whose jobs require strenuous muscular effort and who cannot obtain adequate diets because of local food and eating place limitations would seem to be highly beneficial according to results obtained with a group of university students. It was found that when the students were put on a diet deficient in the B complex such as may be eaten by large numbers of workers, they soon exhibited a lack of pep and appetite, leg pains, and a lower level of work efficiency. The addition of B complex vitamins to the diet restored work output to higher levels and banished the other symptoms.

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